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Prevalence of Hepatitis B, Hepatitis C, and GB Virus C/Hepatitis G Virus Infections in Liver Disease Patients and Inhabitants in Ho Chi Minh, Vietnam

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The prevalence of hepatitis B virus (HBV), hepatitis C virus (HCV), and GB virus C or hepatitis G virus (GBV-C/HGV) infections was determined in 289 patients with liver disease in Ho Chi Minh City and 890 healthy inhabitants of its rural area, Dalat City, Vietnam, respectively. Serum HCV RNA and GBV-C/HGV RNA were detected by reverse transcription–polymerase chain reaction (RT-PCR). HBsAg, HCV antibodies, and GBV-C/HGV RNA were detected in 139 (47%), 69 (23%), and ten (3%) subjects, respectively, often accompanied by elevated serum levels of alanine aminotransferase. HBsAg and HCV antibodies or HCV antibodies and GBV-C/HGV RNA were detectable simultaneously in 8% and 2% of the patients, respectively. In the inhabitants, HBsAg, HCV antibodies, and GBV-C/HGV RNA were found in 51 (5.7%), nine (1.0%), and 11 (1.2%) subjects, respectively. Thus, the prevalence of HBsAg, HCV antibodies, and GBV-C/HGV RNA was significantly higher in liver disease patients than those in the general population. In the samples from 69 patients and nine inhabitants who were seropositive for HCV antibodies, HCV RNA was detectable in 42 (61%) and 4 (44%), respectively. In patients with liver disease, ten belonged to HCV genotype 1a, ten to HCV 1b, three to HCV 2a, four to HCV 2b, and two to HCV 3a by PCR with genotype-specific primers. Nine patients had mixed genotypes, and the remaining four were not classified. Of the GBV-C/HGV RNA-positive individuals, two patients and two inhabitants were positive for HBsAg, while none of the residents had HCV antibodies, although six HCV antibodies (60%) and four HCV RNA (40%) were found in patients. When a phylogenetic tree of GBV-C/HGV was constructed based on the nucleotide sequences, the 21 isolates were classified into at least two genotypes; four isolates belonged to G2, and 17 to G3. The results indicate that in Ho Chi Minh HCV infection prevails with broad distribution of genotypes together with HBV infection among patients with liver disease. This study suggests that GBV-C/HGV infection occurs independently in the two different districts in association with HCV infection. ***J. Med. Virol. 54:243–248, 1998.***

KEY WORDS: epidemiology; Southeast Asia; hepatitis

INTRODUCTION

Infection with hepatitis viruses is prevalent in many parts of Asia. In southeast Asia the carriage of hepatitis B virus (HBV) varies, but is commonly around 15% [Catterall and Murray-Lyon, 1992]. Hepatitis C virus (HCV) is common in some Asian countries [Ohno et al., 1994; Wang et al., 1994; Apichartpiyakul et al., 1994]. Infection with hepatitis viruses appears to be related to a high frequency of liver cirrhosis and hepatocellular carcinoma [Kiyosawa et al., 1990; Takano et al., 1995].

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TABLE I. Prevalence of HBV, HCV, and GBV-C/HGV Markers Among 298 Hospitalized Patients With Liver Disease at Cho Ray Hospital, Ho Chi Minh City*

Hepatitis virus markers	Positivity for the marker(s): No. (%)	Mean age in years (range)	Male/ Female	Mean ALT levels (IU/L) per person tested
HBsAg	139 (46.6)	35 (7–74)	92/47	317/108
HCV Ab	69 (23.1)	44 (18–82)	42/27	190/58
GBV-C/HGV RNA	10 (3.3)	40 (22–66)	6/4	631/7
HBsAg and HCV Ab	23 (7.7)	36 (18–51)	12/11	205/16
HBsAg and GBV-C/HGV RNA	2 (0.7)	30 (24–35)	1/1	NK
HCV Ab and GBV-C/HGV RNA	6 (2.0)	44 (23–66)	4/2	350/4
HBsAg, HCV Ab, and GBV-C/HGV RNA	1 (0.3)	35	1/0	NK

*NK, not known.

GV virus C (GBV-C)/hepatitis G virus (HGV), a single-stranded RNA virus that belongs to the Flaviviridae family, was first identified from the serum of patients with non–A-E hepatitis by Schlauder et al. [1995], Simons et al. [1995a,b], and others with a global distribution [Linnen et al., 1996]. The virus is present in 1–2% of blood donors in the United States, a frequency which is higher than that of HBV or HCV [Linnen et al., 1996]. The possible involvement of GBV-C/HGV has been reported in the etiology of fulminant hepatic failure [Yoshiba et al., 1995; Heringlake et al., 1996].

Hepatitis B and C viruses are transmitted parenterally, in the past typically via blood transfusion, intravenous drug abuse, sexual exposure, and contaminated needles, and the infections commonly cause acute and chronic liver disease. Recent advances in laboratory diagnosis have allowed the differentiation of most cases of non–A-E hepatitis, but the availability of the new procedures in developing countries has been limited by cost.

Although in Ho Chi Minh City of Vietnam the prevalence of hepatitis B surface antigen (HBsAg) in various "at risk" populations ranges from 8.8% among nightclub workers to 62.7% among liver cancer patients [Tran van Be et al., 1993], only limited data on clinical hepatitis have been reported [Tran van Be et al., 1993; Nakata et al., 1993]. We investigated therefore the prevalence of HBV, HCV, and GBV-C/HGV infections in patients with clinically recognized liver disease at Cho Ray Hospital in Ho Chi Minh City, with emphasis on the distribution of HCV genotypes, and the prevalence and genome sequence of GBV-C/HGV. In addition, a survey on the seroprevalence of hepatitis virus–associated markers was carried out in the general population from a rural area near Ho Chi Minh City.

TABLE II. Distribution of the Disease of Patients Diagnosed as Having Hepatitis B and/or Hepatitis C*

Diagnosis	Hepatitis B N = 139 (%)	Hepatitis C N = 48 (%)	Hepatitis B and hepatitis C N = 23 (%)
Acute hepatitis	46 (33.1)	9 (18.8)[a]	6 (26.1)
Chronic hepatitis	29 (20.9)	21 (43.8)	9 (39.1)
Liver cirrhosis	21 (15.1)	12 (25.0)	5 (21.7)
HCC	11 (7.9)	5 (10.4)	2 (8.7)
Asymptomatic carrier	32 (23.0)	1 (2.1)	1 (4.3)

*HCC, hepatocellular carcinoma; Asymptomatic carrier, HBsAg and/or HCV RNA–positive patient with normal values of serum alanine aminotransferase.
[a]Among nine patients diagnosed as having acute hepatitis C, six were seropositive only for HCV Ab with undetectable HCV RNA.

TABLE III. Prevalence of HBV, HCV, and GBV-C/HGV Markers Among 890 Residents of a Rural Area of Ho Chi Minh City*

Hepatitis virus markers	Positivity for the markers(s): No. (%)	Mean age in years (range)	Male/ Female
HBsAg	51 (5.7)	NK	NK
HCV Ab	9 (1.0)	42 (17–77)	4/5
GBV-C/HGV RNA	11 (1.2)	30 (2–64)	7/4
HBsAg and HCV Ab	2 (0.2)	43 (31–54)	1/1
HBsAg and GBV-C/HGV RNA	2 (0.2)	31 (22–40)	1/1

*The residents surveyed consisted of 371 males, 516 women, and three unknown.
NK, not known.

MATERIALS AND METHODS

Patients

Two hundred ninety-eight consecutive patients (178 men and 120 women with a mean age of 37 years, range; 26–71), who were admitted to Department of Infectious Disease at Cho Ray Hospital, Ho Chi Minh City, during 1994 to 1996 and diagnosed with liver disease based on clinical and laboratory findings, were selected randomly for study. In some patients, the diagnosis was confirmed by liver biopsy, surgery, and/or ultrasonography. In addition, the seroprevalence of hepatitis virus markers in a cohort of the general population selected at random living in Dalat City, Lamdong Province, was surveyed in 1996: a total of 890 residents including 371 men, 516 women, and three unknown with a mean age of 28 ranging from 2 to 81 years old. Most inhabitants were born in the area and had rarely traveled. A venous blood sample was taken, and the serum was stored in aliquots at −20°C for transport and storage until tested.

TABLE IV. Characteristics of Ten Liver Disease Patients Seropositive for GBV-C/HGV RNA*

Case No.	Age (years)	Sex	HBs Ag	HCV Ab	HCV RNA	HCV genotype	ALT (IU/L)	Diagnosis
H 13	40	M	–	–	–		20	HCC
H 32	23	F	–	+	–		High	Hepatic coma (fulminant hepatitis)
H 64	48	M	–	+	–		496	Chronic hepatitis
H 67	51	F	–	–	–		NK	Unknown
H 94	35	M	+	+	+	1a	NK	Unknown
H 123	22	M	–	–	–		1412	Acute hepatitis
H 172	54	M	–	+	+	1b	783	Acute hepatitis
H 181	66	F	–	+	+	2b	108	Liver cirrhosis
H 218	40	M	–	+	+	1a + 1b	13	Hepatomegaly (HCV carrier)
H 337	24	F	+	–	–		1,584	Acute hepatitis?

*NT, not tested; NK, not known.

TABLE V. Features of 11 Inhabitants Seropositive for GBV-C/HGV RNA

Case No.	Age (years)	Sex	HBsAg	HCV Ab	HCV RNA
D 81	48	M	–	–	–
D 83	2	F	–	–	–
D 196	37	F	–	–	–
D 346	6	F	–	–	–
D 347	19	M	–	–	–
D 351	40	M	+	–	–
D 360	22	F	+	–	–
D 498	13	M	–	–	–
D 740	34	M	–	–	–
D 752	64	M	–	–	–
D 776	44	M	–	–	–

Biochemical and Hepatitis Virus–Associated Serological Tests

Laboratory tests were carried out on each patient at inclusion in the study. HBsAg and HCV antibodies were examined by reverse passive hemagglutination (SERODIA-HBs, Fujirebio Inc., Tokyo, Japan) and by second-generation passive hemagglutination (Abbott Laboratories, North Chicago, IL) tests, respectively.

Determination of HCV RNA and Genotypes by Polymerase Chain Reaction (PCR)

Serum samples reactive for HCV antibodies were tested for HCV RNA. Nucleic acids were extracted from 100 μl of serum, reverse-transcribed to cDNA and amplified by a two-stage PCR with nested primers deduced from the 5′-noncoding region of the HCV genome [Okamoto et al., 1991].

Okamoto's method [1992, 1993] for HCV genotyping, in which PCR was performed with genotype-specific primers derived from the core protein-coding region, was used. The results are described according to the Simmonds et al. [1993] classification.

Determination of GBV-C/HGV RNA by PCR

Nucleic acids were extracted from 100 μl of serum samples with ISOGEN-LS (NIPPON-GENE, Toyama, Japan) and were converted to complementary DNA (cDNA) with reverse transcriptase (Superscript II, GIBCO-BRL, MD) and antisense primer #G8 (5′-CTATTGGTCAAGAGAGACAT-3′). cDNAs were subjected to the first round of PCR with sense primer #G7 (5′-CAGGGTTGGTAGGTCGTAAATC-3′) and antisense primer #G8. PCR was performed with TaKaRa Ex Taq polymerase (TaKaRa Biochemicals, Kyoto, Japan) for 30 cycles (consisting of denaturation for 20 seconds at 92°C, annealing for 10 seconds at 50°C, and extension for 40 seconds at 70°C). The second round of PCR was carried out for 30 cycles with nested primers: sense primer #G24 (5′-GGTCATCCTGGTAGCCACTATAGG-3′) and antisense primer #G25 (5′-AAGAGAGACATTGAAGGGCGACGT-3′). All primers were deduced from the nucleotide sequences of a 5′-untranslated region of HGV described by GenBank accession number U44402 and U36380.

Nucleotide Sequences of GBV-C/HGV Isolates

The amplified products were cut out from agarose gels for the direct sequencing. Sequencing reactions were performed with AmpliTaq DNA polymerase (Perkin Elmer). Sequences of GBV-C/HGV cDNA were determined by 373S DNA sequencing system (Applied Biosystems, (Foster City, CA).

Statistical Analysis

Results were expressed as mean ± SD and analyzed using the paired and unpaired Student's t-test, χ^2 test, or Fisher's exact test. P values of .05 or less were regarded as significant.

RESULTS

Table I summarizes the prevalence of HBV, HCV, and GBV-C/HGV markers among patients admitted to the hospital with liver disease in Ho Chi Minh City. Of 298 patients, HBsAg, HCV antibodies, and GBV-C/HGV RNA were detected in 139 (47%), 69 (23%), and ten (3%) patients, respectively, often accompanied with elevated serum levels of alanine aminotransferase (ALT). Among the HBsAg-positive patients, 23 (8%) were also positive for HCV antibodies, two for GBV-C/HGV RNA, and the remaining one for both HCV antibodies and GBV-C/HGV RNA, respectively. Coinfection of HCV antibodies and GBV-C/HGV RNA was seen in six (2%) patients. Of 139 HBsAg-positive patients, 46 (33%) were diagnosed with acute hepatitis and jaun-

TABLE VI. Genotypic Distribution of HCV in Liver Disease Patients and Residents in Ho Chi Minh City and Its Rural Area*

	HCV Ab positive: No	HCV RNA Positive: No. (%)	Genotypes: No.										
			1a	1b	2a	2b	3a	1a + 1b	1a + 2b	1b + 2a	1a + 1b + 2b	1a + 2b + 3a	NC
Liver disease patients	69	42 (61%)	10	10	3	4	2	1	3	1	1	3	4
Residents	9	4 (44%)	0	2	1	0	0	1	0	0	0	0	0

*NC, not classified.
With respect to the assay for HCV markers and genotypes, see text.
HCV genotypes were classified according to Simmonds et al. [1993].

dice, 61 (44%) as chronic liver disease including 11 patients with hepatocellular carcinoma (HCC), and the remaining 32 (23%) as asymptomatic carriers of HBsAg with normal serum ALT (Table II). Of 48 patients with hepatitis C, nine had acute hepatitis; six of nine were seropositive only for HCV antibodies without detectable HCV viremia, while 38 patients had chronic liver disease including five patients with HCC, and one asymptomatic carrier of HCV. Twenty-three patients with both hepatitis B and hepatitis C viral markers also had similar disease distribution compared to single infection with HBV or HCV.

In the general population of a rural area near Ho Chi Minh City, HBsAg, HCV antibodies, and GBV-C/HGV RNA were found in 51 (5.7%), nine (1.0%), and 11 (1.2%) subjects, respectively (Table III). Co-infections of HBsAg and HCV antibodies or HBsAg and GBV-C/HGV RNA were noted in two persons in each group.

Positive rates of HBsAg, HCV antibodies, and GBV-C/HGV RNA were significantly higher in patients with liver disease ($P < .0001$, $P < .0001$, and $P < .02$, respectively) than in the general population. In addition, co-infection of HBsAg and HCV antibodies was noted more frequently in patients ($P < .0001$) compared with that of inhabitants. Notably, HCV antibodies and HCV RNA were detected simultaneously in six and four out of ten GBV-C/HGV RNA–positive patients, respectively, and in none of 11 GBV-C/HGV RNA–positive individuals in the general population (Tables IVa, V). Among the 21 viral RNA–positive individuals, two patients and two residents were positive for HBsAg.

There appeared to be no correlation between the frequency of the viral markers tested here and the distribution of age and gender.

Of the samples from 69 patients and nine inhabitants with HCV antibodies, HCV RNA were detectable in 42 (61%) and four (44%), respectively (Table VI). In patients with liver disease, ten were shown to belong to the HCV genotype 1a, ten to HCV 1b, three to HCV 2a, four to HCV 2b, and two to HCV 3a. Nine patients had mixed genotypes; for example, three had HCV 1a + 1b and three had HCV 1a + 2b + 3a. The remaining four samples were not classified. In residents, two had genotype 1b, one had genotype 2a, and the other one had mixed genotypes of 1a + 1b.

A phylogenetic tree of GBV-C/HGV was constructed by the unweighted pair-group method with arithmetic mean based on the nucleotide sequences in the 5′UTR region of the 21 isolates in the present study of Vietnam and six isolates published previously [Linnen et al., 1996; Simmonds et al., 1995; Okamoto et al., 1997; Nakao et al., 1997] (Fig. 1). The 21 isolates were classified into at least two genotypes with evolutionary distance >0.10. Thus, four isolates appeared to belong to G2, while 17 isolates belonged to G3; no isolate was classified into the G1 based on Okamoto et al.'s classification [1997].

DISCUSSION

In Ho Chi Minh City a major cause of liver disease is HBV infection, as expected, and the prevalence of HBsAg in patients with liver disease in this study appears to be consistent with previous reports in which HBsAg rates were 29.0% in hepatitis patients and 62.7% in liver cancer patients, respectively [Tran van Be et al., 1993]. On the other hand, the rate of HBsAg among general populations was lower in this study compared with that of Nakata et al. [1993], who found that the carrier rate of HBsAg was over 10% in all age groups in Ho Chi Minh City and Hanoi. The difference may reflect a particular circumstance of the rural area near Ho Chi Minh City, in that most residents were born in the area and have rarely traveled.

Blood supply in Vietnam depends on commercial donors who are screened for HBsAg, syphilis, and malaria, but not for HCV. Therefore drug users may have donated blood contaminated with HCV. These factors may be responsible for a relatively high prevalence of HCV antibodies among liver disease patients in Ho Chi Minh City. Thus, there is an urgent need to screen all blood donors for HCV. The prevalence of HCV antibodies in the general population was low (1.0%) compared with that reported from Ho Chi Minh City (9%) by others [Nakata et al., 1993]; community-acquired HCV infection may contribute to a high prevalence of HCV antibodies in populations without known risk factors for infection.

Tokita et al. [1994] described that 34 (41%) of 83 HCV isolates from commercial blood donors in Vietnam (79 from Ho Chi Minh) were not classifiable into genotypes 1a, 1b, 2a, 2b, or 3a in contrast to our study in which only 9.5% of HCV from liver disease patients were not classifiable. We observed a wide-ranging distribution of HCV genotypes from patients, and the major genotypes were 1a and 1b (23.8%). These findings were similar to those of Tokita et al. [1994]. In south-

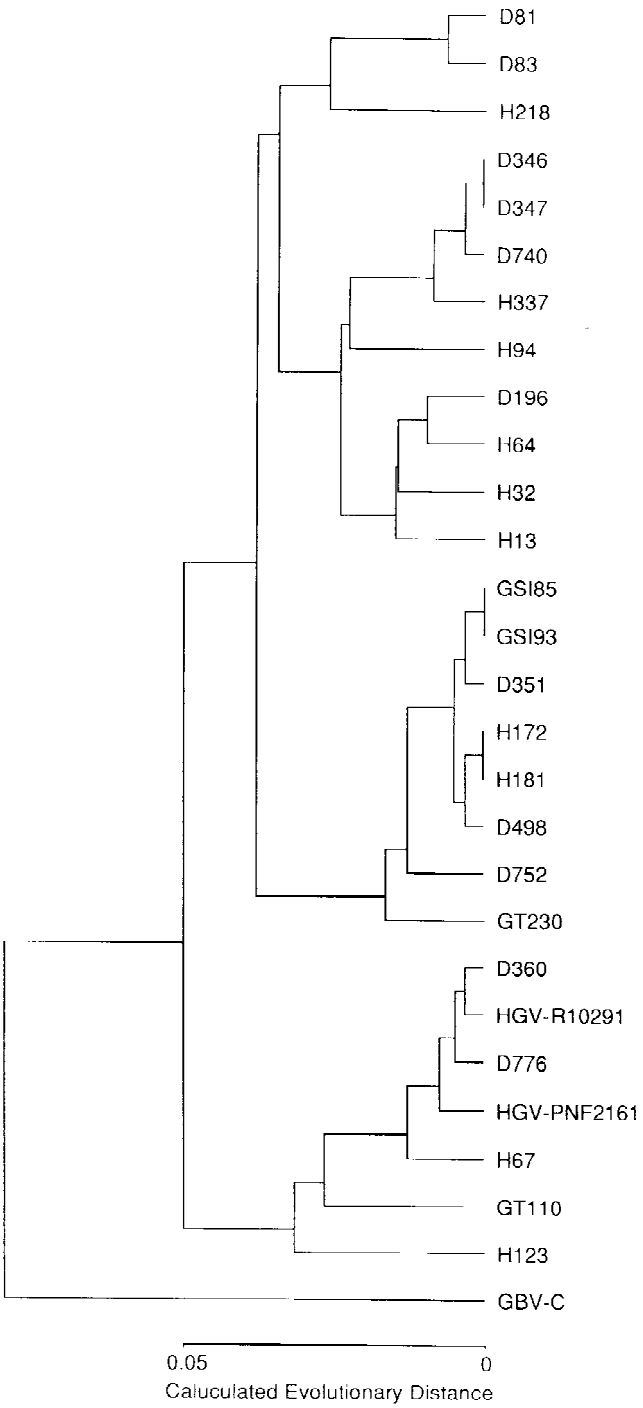

Fig. 1. A phylogenetic tree of GBV-C/HGV which was constructed by the unweighed pair-group method with arithmetic mean based on the nucleotide sequences in the 5′UTR region of the 21 isolates in the present study of Vietnam [ten from hepatitis patients (H218 etc.) of Ho Chi Minh City and 11 from inhantibodiesitants (D81 etc.) of Dalat City] and six isolates published previously. The isolates in Vietnam were classified into two genotypes, G2 and G3 designated by Okamoto et al. Seventeen isolates belonged to G3, Asian type, with GT230, GS185, and GS193. Four isolates belonged to G2, North American type, with HGV-PNF2161, HGV-R10291, and GT110. Each genotype included the isolates of both patients with hepatitis and residents.

east Asia, genotypes 1a, 1b, and 3a predominate according to the literature [Greene et al., 1994; Doi et al., 1996]. Alternatively, HCV RNA positivity rate seemed to be low among both patients and inhabitants positive for HCV antibodies as found in the present study. This implies that past infection to the virus is more common than present exposure, although it is also possible that the primers used here were mismatched to detect HCV RNA in their sera.

It is known that GBV-C/HGV infection can be detected throughout the world, and the frequency of GBV-C/HGV RNA in the patients with acute and chronic liver disease is higher than in blood donors [Linnen et al., 1996; Masuko et al., 1996; Alter HJ et al., 1977: Alter MJ et al., 1977; Wu et al., 1997; Orito et al., 1996; Stark et al., 1996]. In this study, a significant difference between patients and inhabitants was also found in relation to the incidence of GBV-C/HGV RNA in Ho Chi Minh City and its rural area, although the frequency (3.4%) in liver disease patients tended to be low compared with those reported by others (5–40%) [Linnen et al., 1996; Masuko et al., 1996; Alter HJ et al., 1977: Alter MJ et al., 1977; Wu et al., 1997; Orito et al., 1996; Stark et al., 1996; Stark et al., 1996; Aikawa et al., 1996]. Positive rates (1.2%) of the general population for GBV-C/HGV RNA were almost compatible with 0.5–4.0% of those previous studies.

GBV-C/HGV infection was found to be common among patients who were also infected with other hepatitis viruses [Masuko et al., 1996; Orito et al., 1996; Aikawa et al., 1996; Kao 1997; Nakatsuji et al., 1996]. The carrier rate of HBsAg among GBV-C/HGV RNA–positive patients with liver disease and the general population was nearly the same in the present study. Notably, however, none of the inhabitants with GBV-C/HGV RNA had HCV antibodies or HCV-RNA, whereas they were detectable in 60% and 40% of patients with liver disease, respectively. This finding suggests that GBV-C/HGV infection occurred in an independent way among the people living in Ho Chi Minh City and Dalat. To explore this possibility, we conducted an evolutionary analysis of GBV-C/HGV in the nucleotide of the 5′UTR region to analyze the relation with the prevalence of HCV infection between patients with liver disease and the general population. The isolates were classified into two genotypes of GBV-C/HGV, and both genotypes were found in the isolates of both patients and residents.

A high prevalence (5.7%) of GBV-C/HGV was noted among 228 healthy persons in Ho Chi Minh City [Brown et al., 1997]. However, recent reports indicate that GBV-C/HGV is not a hepatitis virus [Alter HJ et al., 1997; Alter MJ et al., 1997], because the studies did not implicate GBV-C/HGV as an etiologic agent of non–A-E hepatitis; persistent infection was common, but most GBV-C/HGV infections were not associated with hepatitis. In addition, GBV-C/HGV did not worsen the course of concurrent non–A-E hepatitis virus infection.

Based on the present findings, further investigation is required to explain these unexpected results in Viet-

nam. The virus can be transmitted by blood transfusion and presumably by other routes.

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